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Elicit of Chamomile (Matricaria chamomilla L.) Extract and Its Antioxidant Activity in Fat-containing Foods

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Abstract

Antioxidants prevent the reaction of free radicals with biomolecules and can remain the nutritional values and physiological properties of foodstuffs. Chamomile (Matricaria chamomilla L.) is a valuable medicinal plant. This study shows the antioxidant effect of Chamomile extract. The antioxidant activity and stability was investigated with three methods, DPPH free radical scavenging system; determine of the peroxide and thiobarbituric acid numbers. The extract antioxidant activity was determined from 0.2 to 1 mg/ml concentrations in crude sunflower oil as a fat-containing food. The antioxidant effects were valuable and rose by increasing the extract concentrations.

Keywords: Chamomile, Extract, Peroxide number, TBA number, Antioxidant, fat-containing foods

Introduction

Chamomile is sometimes known as “the plant doctor”, because it is thought to help the growth and health of many other plants, especially ones that produce essential oils [1, 2]. It is thought to increase production of those oils, making certain herbs, like mints (spearmint, sage, oregano) and basil stronger in scent and flavor [3,4].

Chamomile tea is also thought to be useful to suppress fungal growth [5], for example, misting it over seedlings may prevent damping off. The main chemical components of the chamomile extraction oils are α-pinene, β-pinene, camphene, sabinene, myrcene, 1,8-cineole, y-terpinene, caryophyllene, propyl angelate, butyl angelate, chamazulene, a-bisabolol, bisabolol oxide A, bisabolol oxide B and bisabolone oxide A [6,7]. Also apigenin 7-O-glucoside and various acylated derivatives of apigenin 7-O-glucoside were identified in chamomile [2, 8, 9].

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Oil oxidation is a free radical chain process leading to the deterioration of oil and lipid containing materials [10]. Antioxidant addition is one of the most effective means to retard oxidation [11]. The action of antioxidant depends on its participation in a series of reactions involving radicals [12-14].

Recently, the interest in natural antioxidants has been increased since the application of the most widely used synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) has been questioned because of possible toxic and carcinogenic components formed during their degradation [15-17]. Phenolic compounds are the main class of natural antioxidants [18,19].

Chamomile (*Matricaria chamomilla L.*) is one of the popular ingredients in herbal teas [20-22]. This herb has been traditionally used for medicinal purposes such as selective COX-2 inhibitor with anti-inflammatory activity [23], antimicrobial action [24], antioxidant action [25], antiplatelet action [26], chemopreventive action [27], and so on. Therefore, the research on active constituents in chamomile extract is widely carried out. The conjunction of chamomile with sedative drugs such as analgesics, benzodiazepines, or alcohol may be contraindicated [28-30].

Chamomile (*Matricaria chamomilla L.*) essential oil has been reported as a natural antioxidant [31].

Materials and Methods

Materials

Chamomile (*Matricaria chamomilla L.*) Powder which was obtained from the fresh harvest in a region of Shiraz, Iran. A sample of crude sunflower oil was obtained from Ghoo oil company, Tehran. All chemical compounds and solvents purchased from Merck Company, Germany.

Preparation of Chamomile Extracts

10 g of dried sample were extracted with 100 ml of ratio of 1:1 ethyl alcohol and water with shaking at room temperature for 1h and then heat the mixture at 80 ^{O}C for 30 min. to give an initial extract, the infusion was cooled to room temperature, filtered (fraction I).The residues were extracted with the same process again (fraction II) the two fractions were combined and dried with evaporated under vacuum at 50 ^{O}C and weighed to determine the yield [24, 25].

Sample Preparation

Inhibition effect of the oil substrate was achieved by adding of the certain methanolic solution of the antioxidant to a weighed oil sample. Samples contain 0.2, 0.4, 0.6, 0.8 and 1 mg/ml of

the antioxidant in the crude sunflower oil as a fat-containing food without antioxidant, were prepared. The samples and a control sample, crud sunflower oil without antioxidant, were located in 60 ^{O}C for certain of period times.

The peroxide and Thiobarbituric acid number of samples were measured in 0, 8, 16, 24 and 32 days.

Determination of Antioxidant Activity

Antioxidant activity was determined by the methods to determine of peroxide and thiobarbituric acid numbers.

Peroxide Method

This measurement was accomplished according to the AOCS method. In a beaker 250 ml, added 3g of each samples to 30 ml of a solution of acetic acid and chloroform (1:1) and mixed to prepared a suitable solution, then added 0.5 ml saturated solution of KI and stirred it for one minute, 30 ml stilled water was added and titrated with 0.01 N sodium thiosulphate, the yellowish color was disappeared. Now added 0.5 ml starch glue indicator to the solution, continued the titration until the blue color was disappeared. The peroxide number calculated by this equation:

$$PV = (S\text{-}S_0) \times N \times 1000/W$$

Where S is the amount of consumption thiosulphate for samples, S_0 is the amount of consumption thiosulphate for control sample, N is the thiosulphate solution normalization, W is the weight of samples (g).

Thiobarbituric Acid Method (TBA)

In addition of the peroxide number, the thiobarbituric acid number (TBA) is a complement test to determine of the antioxidant activity. The thiobarbituric acid number measures the Malon dialdehyde (MDA) per one kilogram oil. This measurement was accomplished according to the Sidewell method. In a container 250 ml, dissolved 1 g of each samples to 10 ml of carbon tetrachloride and added 10 ml thiobarbituric acid solution (aqueous solution of 0.67% thiobarbituric acid in the same volume of glacial acetic acid) then stirred it for 2 h and centrifuged for 5 min. with 1000 rpm after this step the aqueous layer was separated and heated in boiling

water bath for 1 h. finally, the absorption values was measured at 532 nm wavelength.
TBA was calculated as bellow equation:

$$E = e/ (d. a)$$

Where e is measurement absorption, d is cell thickness and a is weight of sample (g).

DPPH Method

This measurement was carried out according to the Brand - Williams method. In this method prepared seven samples from 1 to 7 mg/ml of the antioxidant and the free radical of DPPH· reacted with the antioxidant and produced yellowish color and reduced the absorption value in wave length to 517 nm. Then added 0.5ml of various concentrations of extracted oil to 2 ml of 6×10^{-5} methanolic solution of the DPPH· free radical and stay in room temperature for 1h. The absorption of solution was read in 517 nm. The control sample was a solution of 0.5 ml methanol in 2 ml solution of the DPPH·. This test was repeated for 3 times. Radical Scavenging Activity (RSA) was determined in below:

$$\% RSA = [1- ((A_{control} - A_{sample}) / A_{control}) \times 100]$$

Where $A_{control}$ is the control absorption and A_{sample} is the sample absorption. We used from EC_{50} to explain the anti-radically activities which is the percent of the extraction that be able to neutralized 50% of the initial DPPH· free radical.

Statistical Analysis

All determinations were carried out in three triplicate and data were subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses were performed according to the MSTATC software.
Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

Results and Discussion

Figure 1 illustrate the rate of increase in the anti-radically activities and decrease in the remained DPPH· free radical with arise of the Chamomile extract concentrations from 1 to 7 mg/ml. EC_{50} for Chamomile extract is 5.52 ± 0.15 mg/ml.

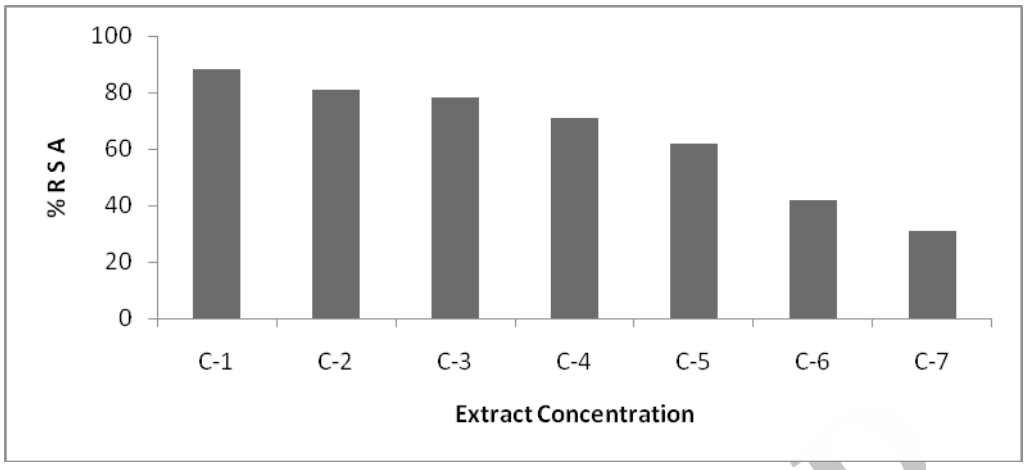

Figure 1. Relationship between radical scavenging activity with Chamomile extract concentration. C-1 to C-7 is extract concentrations from 1 to 7 mg/ml.

Figure 2 shows the rate of decreasing of the remained DPPH· in 60 min. for the various extract concentrations. Radical scavenging activity rise with increasing in the extract concentrations and to be constant after 60 min. There is no descending in the control sample. Therefore this explains the remained DPPH· has inversion that is related to the antioxidant radical scavenging activity.

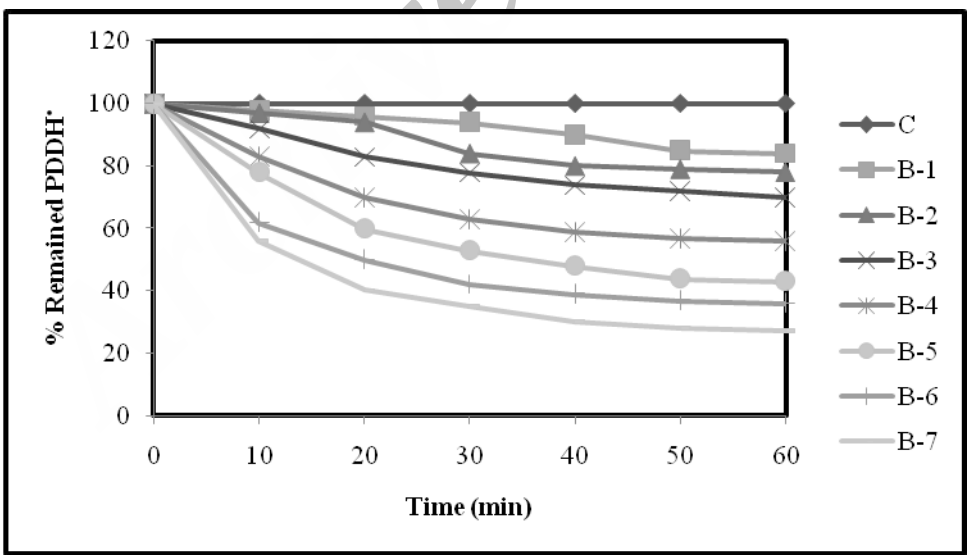

Figure 2. Trend of the reduce percentage of remained PDDH· in 60 min with Chamomile extract concentration. B-1 to B-7 is extract concentrations from 1 to 7 mg/ml.

Table1 show the average of peroxide numbers (meq O_2/kg oil) of the various samples of Chamomile extracts and the control sample in 0, 8, 16, 24 and 32 days.

Table1. The peroxide number (meq O_2/kg) of samples in the five days. Data are the averages of the three repetitions ± standard deviation.

sample	0 day	8th day	16th day	24th day	32th day
C-0.2	0.43 ± 0.12	14.56 ± 0.03	35.65 ± 0.25	45.76 ± 0.30	60.37 ± 0.67
C- 0.4	0.43 ± 0.12	13.59 ± 0.15	32.76 ± 0.65	42.45 ± 0.25	54.45 ± 0.78
C- 0.6	0.43 ± 0.12	13.66 ± 0.35	32.45 ± 0.40	40.86 ± 0.72	52.40 ± 0.12
C- 0.8	0.43 ± 0.12	13.60 ± 0.28	28.51 ± 0.24	39.15 ± 0.12	48.56 ± 0.34
C- 1.0	0.43 ± 0.12	12.43 ± 0.05	26.33 ± 0.06	36.14 ± 0.27	44.20 ± 0.24
Control	0.43 ± 0.12	18.54 ± 0.40	38.45 ± 0.07	61.04 ± 0.55	78.76 ± 0.35

There are the punctual differences among the control sample and the different concentration of extracts and it is clear that the peroxide numbers are depend on the sample concentrations. Increase the concentration makes decrease the peroxide number and increase the antioxidant effects. These numbers illustrate that Chamomile extracts have antioxidant activities. For example Figure 3 illustrates the peroxide number in the 32th day and shows the punctual differences in the various condensations.

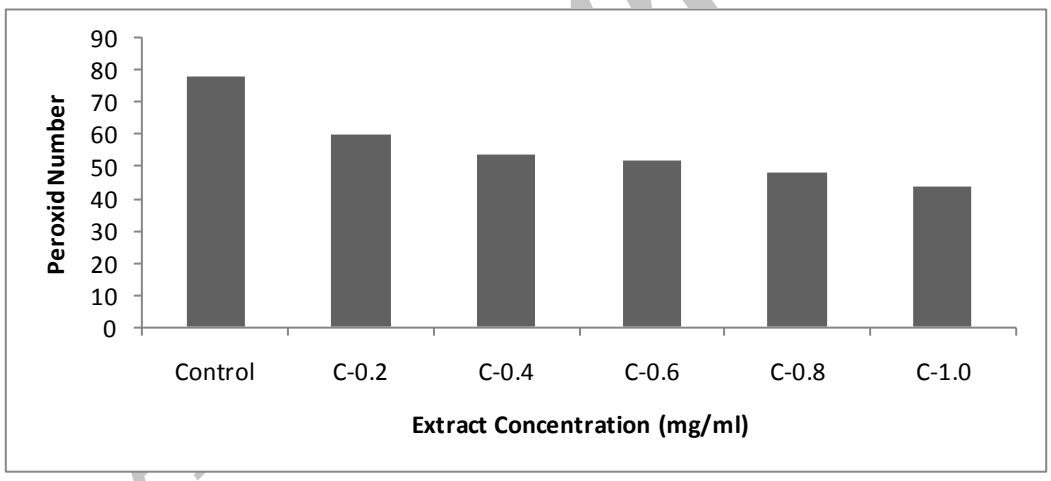

Figure 3. Relationship between the peroxide number with the Chamomile extract concentration.

In addition of the peroxide number, the thiobarbituric acid number (TBA) is a complement test to determine of the antioxidant activity. Table 2 is related to the average of the thiobarbituric acid numbers (meq/kg oil) of the various samples of Chamomile extracts and the control sample in 0, 8, 16, 24 and 32 days.

Table 2. The TBA number (meq MDA/kg oil) of samples in the five days. Data are the averages of the three repetitions ± standard deviation.

sample	0 day	8th day	16th day	24th day	32th day
C-0.2	0.000	0.068 ± 0.01	0.114 ± 0.01	0.216 ± 0.01	0.448 ± 0.01
C- 0.4	0.000	0.059 ± 0.00	0.107 ± 0.01	0.133 ± 0.01	0.432 ± 0.02
C- 0.6	0.000	0.053 ± 0.00	0.089 ± 0.01	0.145 ± 0.00	0.376 ± 0.01
C- 0.8	0.000	0.050 ± 0.01	0.085 ± 0.00	0.110 ± 0.02	0.269 ± 0.01
C- 1.0	0.000	0.041 ± 0.01	0.073 ± 0.00	0.129 ± 0.01	0.230 ± 0.00
Control	0.000	0.093 ± 0.02	0.119 ± 0.02	0.243 ± 0.03	0.586 ± 0.02

There are the punctual differences among the control sample and the different concentration of extracts. The TBA numbers are depend on the sample concentrations such as the peroxide numbers and increase the extract concentration makes decrease the TBA number and increase the antioxidant effects. These numbers illustrate that Chamomile extracts have antioxidant effects. To more realization the results of the 32th day are explained in the Figure 4.

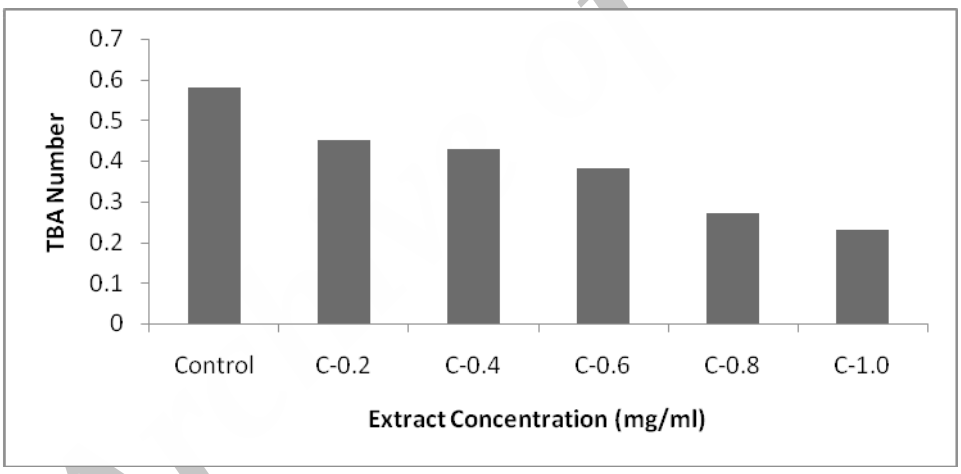

Figure 4. Relationship between the TBA number with the Chamomile extract concentration.

This chart illustrates the TBA number in 32th day and shows the punctual differences in the various condensations. According to the results, this plant has shown antioxidant activity.

Conclusion

The results of this study show that Chamomile (*Matricaria chamomilla L.*) extract at 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml concentration in fat-containing food has antioxidant activity.

According to these studies radical scavenging activity rise with increasing in the extract concentrations and increase the concentration extracts make decrease the peroxide and TBA numbers and increase the antioxidant activities.

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